JOURNAL OF AGRICULTURAL RESEARCH

VOL. 68 WASHINGTON, D. C., APRIL 1, 1944 No. 7

BLISTER SPOT, A BACTERIAL DISEASE OF APPLE[1]

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INTRODUCTION

Blister spot, a relatively inconspicuous disease of apples, was first described briefly by Rose (*27*) [3] in 1916. In a later more detailed account (*28*) he named the causal organism *Pseudomonas papulans* (*Phytomonas papulans* (Rose) Bergey et al. (*4*)). Subsequently various reports of this and similar diseases appeared, but certain phases of the life history and physiology of the causal organism remained uninvestigated. Herein are reported the results of a detailed study of the physiology and pathogenicity of the blister spot organism, in comparison with other organisms, and of the pathological histology of the host. A description of the disease, which may be confused by the unaided eye with minute infections of the apple scab fungus, the known distribution of the disease, and notes on varietal susceptibility are also given.

REVIEW OF LITERATURE

After isolating the causal organism of the blister spot disease, Rose (*28*) reproduced the disease on a number of apple varieties by means of needle punctures or by hypodermic injections, but all attempts to reproduce it by inoculations on nonwounded fruits were unsuccessful. Rose described the morphological and physiological characters of the organism, which was a Gram-negative, white bacterium, producing green fluorescence in certain media. He also undertook a study of a disease that he called rough, or scurfy, bark disease of apple, the active stage of which is characterized by loosening and sloughing of the outer bark. He was successful in isolating a number of species of bacteria from the bark, and he concluded from certain physiological studies that these organisms were closely related to the blister spot organism (*28*). He obtained the typical blister spot disease of apples with two types of the scurfy bark organisms and thought that possibly all were varieties of one species; he suggested, however, that more work on the problem was necessary to clear up this point.

No further mention of the blister spot disease was found in the literature until 1924 when Rhoads (*25*), in an article on apple measles, stated that the role of *Phytomonas papulans* as a causal agent of scurfy bark, as previously suggested by Rose, was very doubtful. His

[1] Received for publication, February 2, 1943. The subject matter of this paper was presented as a thesis to the Graduate School of the University of Illinois in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] The writer gratefully acknowledges the advice, encouragement, helpful criticisms, and suggestions of H. W. Anderson, under whose direction this work was done. He is indebted also to J. W. Roberts, of this Division, for helpful suggestions throughout the work and to H. H. Thornberry and J. C. Dunegan for criticism of certain parts of the manuscript.

[3] Italic numbers in parentheses refer to Literature Cited, p. 296.

attempts to isolate a causal organism from the pustular and scurfy types of measled apple bark were unsuccessful. He stated further that he was unable to find blister spot on apples in the orchards at Mountain Grove, Mo., as earlier described by Rose (*28*).

In 1927 Hopkins [4] published an account of a bacterial disease of apple trees occurring in the Elgin district of South Africa. When he compared the results of his physiological studies of the Elgin organism with two strains of *Phytomonas papulans* (one strain from bark, a slow liquefier of gelatin, and another, a rapid liquefier) and *Erwinia amylovora* (Burr.) Winslow et al., he concluded that the cultural characters of the Elgin organism and of the two strains of *Ph. papulans* clearly indicated that each was a distinct species. He made no attempt to obtain infection on fruits with the Elgin organism, nor is there any record of his having obtained infection on twigs or wood with *Ph. papulans*.

In 1931 Lacey and Dowson (*20*) described a disease of seedling apple trees characterized by cracks in the bark, horseshoe-shaped or circular in outline. Scattered among these were a number of small, raised swellings, or blisters. In most cases isolation from tissue under or within these blisters yielded almost pure cultures of a bacterial organism. When this organism, described as the Wisley strain, was inoculated into apple stems and twigs, lesions similar to those resulting from natural infections developed. In another series of experiments strains of this organism were inoculated on apples in various stages of development, from young to almost ripe fruit, but every attempt to induce infection failed. Inoculation of apple flowers also failed to produce infection. Immature apples and pears were inoculated and kept in moist chambers in the laboratory, but they, too, failed to develop the disease. Lacey and Dowson concluded that the organism which they described is identical with *Phytomonas papulans*, the cause of scurfy bark canker in America. They suggested that *Ph. papulans* should be considered a weak parasite, producing disease only when some physiological condition renders the trees susceptible to attack.

In 1932 Rosen (*29*) announced the discovery of a new pear and apple disease in North America simulating fire blight and having as its etiologic agent a pathogen belonging to the group typified by *Phytomonas barkeri* (*5*), *Ph. nectarophila* (*9*), and *Pseudomanas prunicola* (*37*).

In 1932 also Clara (*8*) published a brief account of a new pear disease caused by a green fluorescent bacterium. He noted that it was closely related to, but not identical with, *Phytomonas syringae* and he regarded it as a new species, which he named *Ph. utiformica*.

In 1934 Roberts (*26*) reported the isolation of a bacterium similar to *Phytomonas papulans* from early lesions associated with a disease which he had previously described as apple target canker. Inoculation with the organism on the variety Delicious produced lesions typical of the early stage of the disease. The results, however, were not conclusive, since similar lesions also appeared on the noninoculated checks. Roberts was of the opinion, however, that apple measles, target canker, and the rough bark disease might prove identical,

[4] HOPKINS, C. J. AN APPLE DISEASE OCCURRING IN THE ELGIN DISTRICT. So. Africa Dept. Agr. Sci. Bul. 61, 17 pp., illus. 1927. [Processed.]

since their early symptoms were similar and what appeared to be the same bacterial species had been isolated from early stages of each.

Also in 1934 Dunegan (*12*) tested the susceptibility of the peach to artificial inoculation with *Phytomonas syringae*, *Ph. prunicola*, *Ph. mors-prunorum*, *Ph. papulans*, a bacterium from apple target canker, and an undetermined bacterial organism from a leaf spot of Italian prune. His studies showed that peach foliage was susceptible to these bacteria when they were actually introduced hypodermically into the tissues of leaves. There was a marked effect on the chloroplasts in the immediate vicinity of the point of inoculation and a stimulation in the formation of anthocyanin pigment in the surrounding regions.

In 1936 Wilson (*35*) reported the results of studies to establish the relation between a canker and blossom blast of pear and the bacterial canker of stone fruit trees. Besides the two strains of stone fruit organisms (*Phytomonas cerasi* and *Ph. cerasi* var. *prunicola*) and bacteria from pear limb cankers and blossom blast, his pathogenicity and cultural studies included the following organisms: *Ph. utiformica*, *Ph. papulans*, *Ph. citriputeale* (*Ph. citriputealis*), cultures obtained from pear blossoms in Arkansas and from limb canker of apple in California, and *Erwinia amylovora*. Wilson's inoculation and cultural tests supported the view that *Ph. utiformica*, *Ph. citriputeale*, and the bacterium isolated from pear blossoms in Arkansas were identical with the stone fruit organisms *Ph. cerasi* and *Ph. cerasi* var. *prunicola*. Wilson concluded that the bacterium isolated by Roberts from target cankers and provisionally designated as *Ph. papulans* was an unrelated species. From a later study of *Ph. syringae* and related organisms Wilson (*36*) concluded that *Ph. cerasi*, *Ph. utiformica*, and *Ph. prunicola* are synonymous with *Ph. syringae*.

THE BLISTER SPOT DISEASE

SYMPTOMS

Blister spot of apples is a relatively inconspicuous disease. Infections on fruits first noted in early June appear as darkened or water-soaked areas, often around lenticels. Later there are formed papillalike swellings or blisters, at first light in color but becoming brown or black. As pointed out by Rose (*28*), an occasional spot may develop into a larger irregularly lobed spot (fig. 1, *A* and *B*). In still later stages the epidermis over the blister dies and often cracks loose from the surrounding healthy tissue (fig. 2, *A*). This blister type with the accompanying darker color is the most conspicuous stage of the disease and by the unaided eye may be confused with minute infections of the apple scab fungus, *Venturia inaequalis* (Cke.) Wint. The disease at this stage is more conspicuous on yellow varieties such as Golden Delicious and Yellow Transparent than on highly colored ones such as King David, Jonathan, and Arkansas Black.

Close observations during the past 2 years and many isolations from suspected lesions have yielded no definite evidence that blister spot occurs naturally on leaves or twigs. A number of isolations from leaf spots of unknown origin yielded bacteria which resembled the blister spot organism in certain physiological and biochemical reactions. A discussion of this phase of the problem occurs on page 286.

DISTRIBUTION AND ECONOMIC IMPORTANCE

Blister spot has been reported from Missouri,[5] Arkansas,[6] Pennsylvania,[7] Virginia,[8] Indiana,[9] and Illinois.[9] The original reports indicated that this disease caused considerable damage to susceptible varieties of apples at Mountain Grove, Mo., in 1916 and 1917. In 1938 the writer observed that 30 percent of the fruits in one of the experimental spray plots were affected with one or more blister spots. Zundel [10] reported in 1938 that in one orchard in Quincy, Franklin County, Pa., 20 percent of the apples had one or more blister spots. In 1939 blister spot was found at Mountain Grove on a greater number of varieties than in 1938, but the infection was less severe. Groves [11] reported it as prevalent in northern Virginia in 1939. In 1940 the disease was present in all the apple scab experimental spray plots at Mountain Grove; in certain plots 15 to 20 percent of the fruit showed infection. In 1940 the disease was seen for the first time at

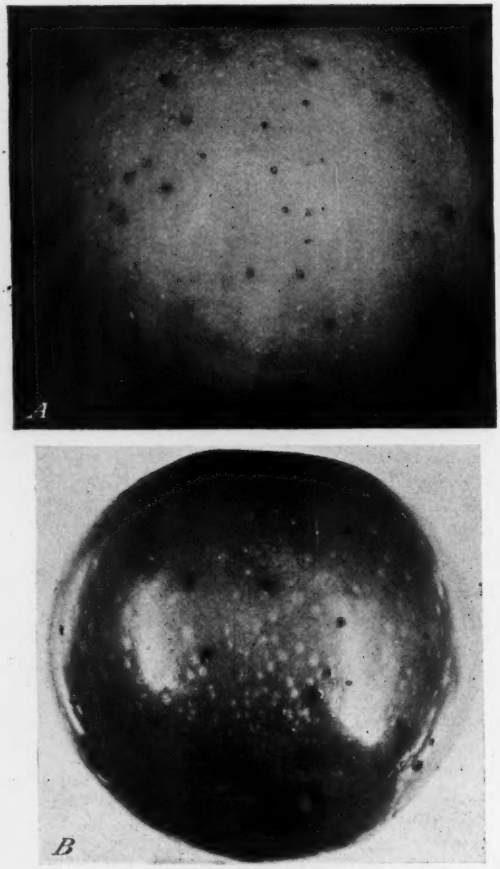

FIGURE 1.—*A*, Blister spot on Rome Beauty apple, × 1; *B*, young blister spot infections on Golden Delicious apple, × 1.

[5] SMITH, M. A. BLISTER SPOT ON ROME BEAUTY APPLES IN MISSOURI. U. S. Bur. Plant Indus., Plant Dis. Rptr. 22: 354. 1938. [Processed.]

[6] HUMPHREY, H. B., and WOOD, J. I. DISEASES OF PLANTS IN THE UNITED STATES IN 1933. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 86: 1–107, illus. 1935. [Processed.] (See p. 33, report on blister spot caused by *Bacterium papulans*, by J. C. Dunegan.)

[7] ZUNDEL, G. L. BLISTER SPOT OF APPLES IN PENNSYLVANIA. U. S. Bur. Plant Indus., Plant Dis. Rptr. 22: 377. 1938. [Processed.]

[8] NANCE, N. W. DISEASES OF PLANTS IN THE UNITED STATES IN 1939. U. S. Bur. Plant Indus., Plant Dis. Rptr. Sup. 128: 210–378, illus. 1941. [Processed.] (See p. 308, report on blister spot, caused by *Phytomonas papulans*, by A. B. Groves.)

[9] Correspondence.

[10] See footnote 7.

[11] See footnote 8.

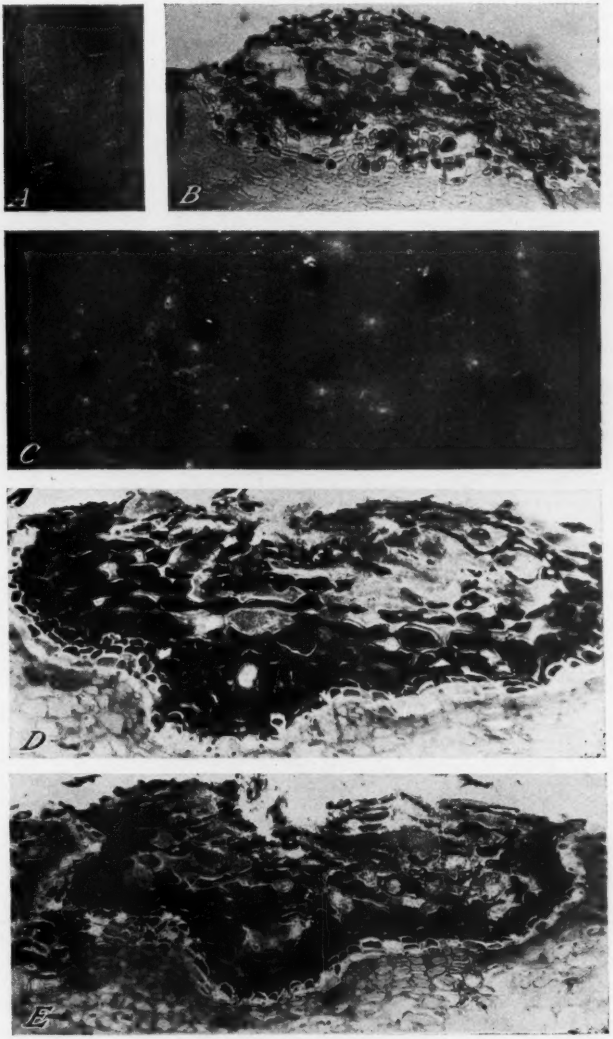

FIGURE 2.—*A*, Blister spot lesions, × 15. Note that the dead tissue has been separated from the surrounding tissue. *B*, Cross section through a blister spot lesion, × 160. Note the raised pustular spot. *C*, Recent infection of an apple by the blister spot pathogen, × 5.5. *D* and *E*, Cross sections through blister spot lesions showing masses of bacteria throughout the host tissues (*D*) and between the diseased tissue and the phellogen layer (*E*), × 200.

Elsberry, Mo., and in other orchards in the central and southeastern parts of the State.

Blister spot is relatively inconspicuous because of the smallness of the usual type of lesions. In years of light infection most of the apples on a tree may be entirely free of the disease and others may have only a few spots. When infections are more prevalent, the lesions may vary from few to many per fruit. Lesions on highly colored fruit may easily be overlooked.

During the past 3 years the writer has not observed any instance of commercially important damage to the crop. The apparent increase in the number of varieties affected by this disease at Mountain Grove during the past 2 years suggests a possible increase in severity of infection at some future time if conditions should be particularly favorable. At present the disease is considered of minor economic importance.

VARIETIES [12] NATURALLY INFECTED

Blister spot has been found under natural conditions only on apple fruits. In 1915 the disease was reported on Hawley, Ishewood, Melon, and Norfolk Beauty. In 1916 it was found on Benoni, Blue Pearmain, Duling, Early Ripe, Hawley, Higginbotham, Isham, Ishewood, Jonathan, Klondike, Lansingburg, Melon, Norfolk Beauty, Red Astrachan, Rock Pippin, White Pippin, Yellow Transparent, and Victuals and Drink. During the past 2 years the writer has observed blister spot on the following: Arkansas Black, Benoni, Black Twig, Carson, Colton, Delicious, Golden Delicious, Jonathan, King David, Liveland, Maiden Blush, Red Astrachan, Rome Beauty, Stayman, Turley, Twenty Ounce, White Winter Pearmain, and Willow Twig.

PATHOLOGICAL ANATOMY OF AFFECTED FRUIT

Small portions of fruit containing blister spots were killed and fixed in a mixture of formalin, acetic acid, and alcohol, embedded, and sectioned in the usual manner. The ethyl alcohol-xylol-paraffin method and the *n*-butyl alcohol-paraffin method of Zirkle (*38*) were used. Occasionally freehand sections of fresh material were made.

Microtome sections 6μ to 10μ thick were stained with basic fuchsin, carbol fuchsin, or gentian violet, with orange G as a counter stain. The thionin-orange-G staining technique, as reported by Stoughton (*33*), was used also and proved to be the most satisfactory for photomicrographs.

Cross sections prepared from blister spots show papillalike swellings on the surface (fig. 2, *B*). The organism gains entry through wounds or lenticels. On recently infected fruits the disease appears macroscopically as spots with water-soaked margins (fig. 2, *C*). Freehand sections of spots collected in the early summer (June 15) often show the cuticle immediately surrounding the blister spot to be unbroken. Immediately beneath the cuticle the host cells are dead.

[12] The nomenclature for the apple varieties listed in this paper is largely that of Beach et al. (*3*), Hedrick (*16*), and Downing (*10*). A few varieties not listed in these publications are American varieties that are not often grown commercially. Hedrick's nomenclature is followed also for some of the other fruit varieties.

Serial cross sections show the diseased area to extend often as much as 0.4 mm. below the cuticle, but as a rule the lesions are not so deep. Stained sections show the bacteria to be present intercellularly throughout the region of the papule. Immediately beneath the diseased area a phellogen layer from 3 to 5 cells in thickness develops (fig. 2, *D*). In some sections the invading organism is seen to have broken through this layer, but invariably it is again walled off by a layer of phellogen which effectively bars it from further extension.

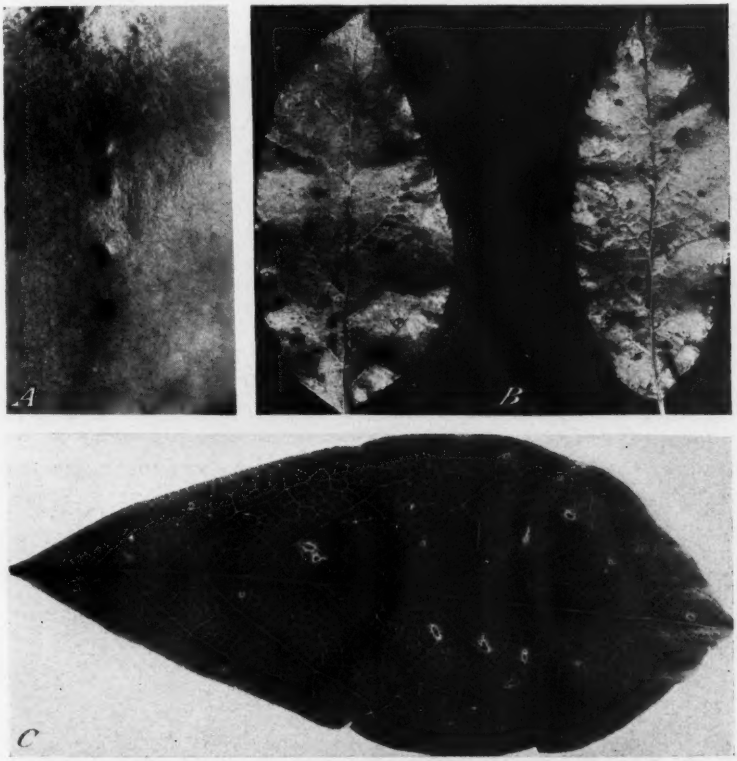

FIGURE 3.—*A*, Artificial infection (needle-puncture inoculation) of Red Astrachan apple by the blister spot organism, × about 2. *B*, Bacterial spot disease on Rome Beauty apple leaves, × about 1. *C*, Bacterial spot disease on leaf of × *Magnolia soulangeana*, × about 1.

Pockets composed of phellogen layers of cells surrounding masses of bacteria are found throughout many of the lesions; it is presumed that these formations are due to the counteraction of the host against bacterial invasion (fig. 2, *E*). Sections of artificially infected tissue show characteristics similar to those of naturally infected tissue. However, it has been observed that when fruits have been artificially inoculated in the laboratory, a definite swelling occurs in the immediate vicinity of the needle puncture, but blisters such as one observes

in the naturally infected fruit are not formed (fig. 3, *A*). In later stages of natural lesions the epidermis over the blister becomes black and dies. As a rule when this occurs, the injured epidermis cracks loose from the surrounding healthy portion (fig. 2, *A*). Where there is no disruption of the epidermis the blister spot may become larger and the lesion may extend outward in various directions, but this condition is rarely observed. Most of the infections in late summer are of the typical blister type.

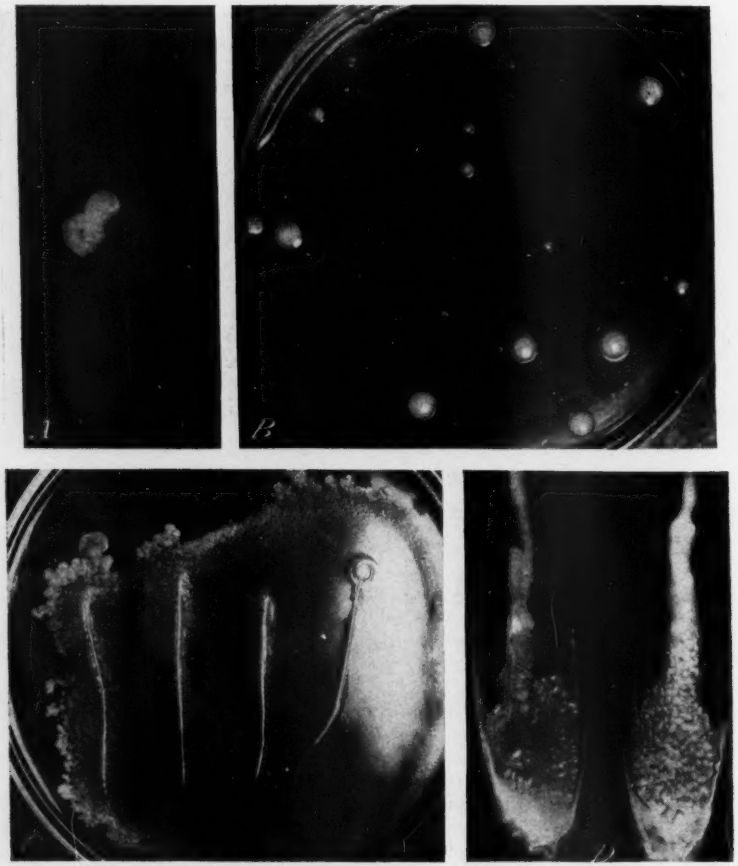

FIGURE 4.—*A*, Bacteria oozing from blister spot planted on beef-infusion agar, × 1. *B–D*, Typical growth of the blister spot organism, × 1: *B*, After 5 days on beef-infusion agar; *C*, after 4 days on Patel's medium; *D*, after 48 hours on beef-infusion-glycerin agar.

SOURCE OF ORGANISMS STUDIED

The blister spot organism is easily isolated from infected apples by sterilizing the surface with 80 percent alcohol, removing a papilla

with a sterilized scalpel, dropping it into a tube of sterile water, and making dilution plates after the bacteria have diffused into the water. Colonies appear in 36 to 48 hours, usually in pure culture (fig. 4, *B*). Isolations may also be made by placing the sterilized papillae directly in poured agar plates (fig. 4, *A*).

Isolations from blister spots have been made from the first appearance of infection in June until mid-September. The percentage of successful isolations from old spots is materially decreased, particularly from those from which the blister has sloughed off, leaving no protection against desiccation. The occurrence of small and shallow blisters seems to indicate that the blister spot organism is a relatively weak parasite.

Rose (*28*) isolated a number of organisms from rough, or scurfy, apple bark. These apparently belonged to the green fluorescent group of bacteria. One of them appeared to have all the cultural characteristics of the blister spot pathogen and when inoculated into healthy apples produced typical blister spots. The writer has made a careful search during the past 2 years for this scurfy bark manifestation. Isolations have been made from cankers of many types, but none has yielded an organism resembling that originally mentioned by Rose.

During the past 2 years a leaf spot characterized by round to irregular purplish areas, located generally in the vicinity of the midvein or veinlets, has been observed often on the leaves of the Rome Beauty apple (fig. 3, *B*). At first this type of spotting was confused with that produced by early infections of *Venturia inaequalis*, which it somewhat resembles. Further examinations of small sections cut in water drops showed masses of bacteria oozing from them in a high percentage of cases. Isolations from such spots often yielded pure cultures of a bacterium which proved to belong to the green fluorescent group. Inasmuch as the blister spot organism had not been reported previously as occurring on apple foliage, it seemed important to make physiological and pathological tests to determine whether any relationship might exist between the blister spot organism and this bacterium from an undescribed apple leaf spot.

During May 1940 the writer also observed a leaf spot on the foliage of × *Magnolia soulangeana* Soul. The spots were round to irregular, water-soaked, ranging in size from 1 to 3 mm., and most apparent on the upper leaf surfaces (fig. 3, *C*). Large numbers of bacteria could be seen oozing from sections of tissue placed in water. A high percentage of isolation cultures from such material yielded a bacterium which later produced a green fluorescence in the beef-infusion agar used as the culture medium. Since this organism from magnolia foliage belongs to the green fluorescent group of bacteria, it seemed important to study it.

The writer's investigations, intended to supply more complete information about the essential physiological characteristics of the blister spot organism than is furnished in the original characterization, included 18 blister spot isolates, 3 isolates of *Phytomonas syringae* (Van Hall) Bergey et al., 2 isolates of the target canker organism provisionally thought to be *Ph. papulans*, and 1 isolate each of the bacteria from the apple and magnolia leaf spots mentioned herein.

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Of the 18 isolates of the blister spot organism, 2 were obtained in 1938, 13 in 1939, and 3 in 1940; 1 was from Arkansas and the rest from 4 localities in Missouri. All were originally isolated from apple fruits of several varieties; 7 had been reisolated after artificial inoculation into apple, plum, tomato, or lemon. Cultures of *Phytomonas syringae* from lilac (*Syringa vulgaris* L.), designated as SI–1 and SH–2 and obtained from Mary K. Bryan, formerly of this Division, had been received originally from Illinois and the Netherlands, respectively; the third isolate designated NY (New York strain) was obtained from W. H. Burkholder, Ithaca, N. Y. Target canker isolates T–62 and T–64 from apple were supplied by J. W. Roberts. The magnolia leaf spot organism was designated MG–19, and the one from Rome Beauty apple leaves was called A–20.

MORPHOLOGY AND STAINING REACTIONS OF BLISTER SPOT AND RELATED ORGANISMS

For uniform determination of form and size of the various isolates smears were made from cultures in beef-extract peptone broth of pH 6.8 which had been incubated for 24 hours at 25° C. Maneval's (*22*) method of negative staining was employed in an attempt to avoid distortion in shape or in size such as might have resulted from the application of a direct stain. In staining flagella the Casares-Gil method, as published by Plimmer and Paine (*24*) and given by the Society of American Bacteriologists (*32*), was employed. The method of Maneval (*21*) was also used. For Gram-staining Hucker and Conn's modification of the ammonium-oxalate-crystal-violet method (*17*, *18*) and Kopeloff and Beerman's modification (*19*) were used. The Ziehl-Neelsen method of acidfast staining was used. The Anthony method (*1*) of capsule staining was used. In table 1 the blister spot organism is compared with the other organisms studied.

PATHOGENICITY OF BLISTER SPOT AND RELATED ORGANISMS

The pathogenicity of the blister spot organism was first demonstrated by Rose (*27*) in 1916. Using subcultures from single colonies, he reproduced the disease on six varieties of apples. The fruits were inoculated while on the trees and were bagged after inoculation. The incubation period averaged 14 days, but some fruits failed to show signs of the disease until after 18 to 25 days. Some strains of the organism infected all the varieties tested and others only one or two. The pathogenicity studies carried on by the writer in 1939 and 1940 are described herein.

FIELD INOCULATIONS

In 1939 inoculations of apple fruits, twigs, and leaves were made by four methods: (*a*) By means of needle punctures, a water suspension of the organism being introduced into a shallow puncture under the epidermis; (*b*) by hypodermic injection of a water suspension of the organism under the epidermis; (*c*) by needle puncture of fruits which had first been atomized with a water suspension of the organism; and (*d*) by atomizing a culture of the organism in water suspension on the surface of nonwounded fruits or leaves. In the needle-puncture inoculation three wounds were made in each twig or fruit. Inoculation of twigs was made by piercing the bark tangentially and then

TABLE 1.—*Comparison of morphology and staining reactions of blister spot and related organisms*

Organism and strain	General description	Size	Capsules demonstrated	Spores	Polar flagella	Gram-negative	Effective stains	Acidfast
Blister spot	Motile rods with rounded ends, usually occurring singly; occasionally in pairs or short chains.	0.8–2.5×.8 μ	No	No	1 to several	Yes	Carbol fuchsin, crystal violet; methylene blue.	No.
Phytomonas syringae	do	1.3–1.9×.5	No	No	do	Yes	Crystal violet; methylene blue.	No.
Target canker isolate (T–62).	Short rods with rounded ends, usually occurring singly.	1.6–1.9×.3	No	No	Nonmotile	Yes	do	No.
Target canker isolate (T–64).	Short rods with rounded ends, occurring singly or in pairs; sometimes in chains.	1.5–2.6×.5	No	No	Not demonstrated	Yes	do	No.
Magnolia isolate (MG–19).	Short motile rods with rounded ends, usually occurring singly.	1.2–1.7×.6	No	No	1 to several	Yes	do	No.
Isolate from apple leaf spot (A–20).	Short motile rods, usually occurring singly; sometimes in pairs or chains.	1.5–2.7×.5	No	No	do	Yes	do	No.

injecting a water suspension of the organism into the opening in the bark. Inoculation of leaves was made by spraying noninjured leaf surfaces with a water suspension of the organism or by scratching either the upper or the lower leaf surface with a hypodermic needle and at the same time introducing a water suspension of the organism. After fruits, either wounded or nonwounded, were inoculated, they were wrapped in a thin layer of moist cotton and then covered with a layer of Parafilm, which was allowed to remain for 24 hours after the inoculation. The points at which the needle punctures were made in twigs were covered by a layer of Parafilm; this was sufficiently elastic to allow for growth expansion and at the same time to give an adequate covering. The inoculated leaves were enclosed in a cellophane bag for a period of 24 hours. In all cases an adequate number of sterile-water (check) inoculations were made in comparable fruit, leaves, or twigs of the same trees.

In 1940 the methods were the same, except that method *c* was not used.

In 1939 inoculations were begun in May and continued at intervals until October; inoculations were begun again in April 1940 and continued until September. The results are summarized in table 2.

TABLE 2.—*Summary of field inoculations with blister spot and other organisms, Mountain Grove, Mo., 1939 and 1940*

Organism and strain	Year and host	Varieties inoculated	Part inoculated	Inoculations made	Method of inoculation [1]	Average period in which infection occurred	Varieties infected	Inoculations resulting in infection (all varieties inoculated)
Blister spot:	*1939*	*Number*		*Number*		*Days*	*Number*	*Percent*
	Apple	8	Fruit	140	*a*	21	5	19
	do	2	do	30	*c*	26	2	23
	do	3	do	70	*d*	26	3	4
	do	1	Leaf	10	*b*			0
	do	2	Twig	30	*a*	36	2	40
	Cherry	4	do	60	*a*	28	2	10
No. 1	Pear	1	do	15	*a*	28	1	33
	do	1	do	15	*b*			0
	Peach	1	do	15	*a*			0
	do	1	Leaf	15	*b*	41	1	13
	1940							
	Lilac	1	Shoot	15	*a*	12	1	40
	Apple	1	Leaf	15	*b*			0
	1939							
	Apple	9	Fruit	165	*a*	19	9	57
	do	1	do	15	*c*	25	1	13
	do	1	do	30	*d*	14	1	13
	do	3	Leaf	75	*b*	14	3	32
No. 2	do	2	do	30	*d*			0
	do	2	Twig	45	*a*	41	2	16
	1940							
	Apple	1	Fruit	20	*a*	22	1	80
No. 7	Apple	1	Leaf	20	*d*			0
	Apple	1	Fruit	15	*d*	21	1	73
	do	1	do	15	*a*	23	1	26
No. 9a	do	1	Twig	15	*a*	32	1	13
	Lilac	1	Shoot	15	*a*	10	1	60
	Pear	1	Twig	15	*a*	26	1	33
	Apple	1	do	20	*a*	22	1	45
No. 10	Lilac	1	Shoot	15	*a*	11	1	26
	Apple	1	Twig	15	*a*			0
No. 12	do	1	Fruit	15	*d*	20	1	40
	Plum	1	Twig	15	*a*	21	1	13
No. 13	Apple	1	Fruit	15	*a*	23	1	13
	Lilac	1	Shoot	15	*d*			0

[1] See p. 278 for description of methods of inoculation.

TABLE 2.—*Summary of field inoculations with blister spot and other organisms, Mountain Grove, Mo., 1939 and 1940*—Continued

Organism and strain	Year and host	Varieties inoculated	Part inoculated	Inoculations made	Method of inoculation	Average period in which infection occurred	Varieties infected	Inoculations resulting in infection (all varieties inoculated)
	1939	*Number*		*Number*		*Days*	*Number*	*Percent*
	Apple	1	Leaf	15	*a*			0
	do	1	Fruit	20	*d*	20	1	20
	do	1	do	15	*a*	21	1	66
	do	1	Leaf	15	*b*	22	1	46
No. 14	Lilac	1	do	15	*b*	11	1	46
	Magnolia	1	do	20	*b*	7	1	50
	do	1	do	20	*d*			0
	do	1	Petiole	20	*a*	6	1	60
	Plum	1	Twig	15	*a*			0
No. 15	Apple	1	do	15	*a*			0
	Apple	2	do	30	*a*			0
No. 16	do	1	Leaf	20	*b*	23	1	30
	Lilac	1	Shoot	15	*a*	12	1	46
No. 17	Apple	1	Fruit	15	*a*	20	1	33
No. 18	Apple	1	Twig	15	*a*	26	1	40
	do	1	Fruit	15	*a*			0
Phytomonas syringae:	*1939*							
	Apple	7	do	70	*a*			0
	do	2	do	20	*d*			0
SI-1	do	1	Leaf	20	*b*			0
	do	1	do	10	*d*			0
	do	1	Twig	10	*a*			0
	Cherry	4	do	40	*a*			0
	1940							
	Apple	2	Fruit	30	*a*	18	1	20
	do	2	do	30	*d*			0
	do	1	Twig	15	*a*	35	1	13
NY	Lilac	1	Leaf	15	*b*	15	1	80
	do	1	Petiole	15	*b*	15	1	80
	do	1	Shoot	15	*a*	15	1	66
	Plum	1	Twig	15	*a*	32	1	33
Target canker:								
	Apple	2	Fruit	60	*a*			0
T-62	do	2	do	30	*d*			0
	do	2	Twig	60	*a*			0
	Apple	2	Fruit	50	*a*			0
T-64	do	2	do	35	*d*			0
	do	2	Twig	40	*a*			0
	Apple	3	Fruit	60	*a*	19	2	27
	do	2	do	45	*d*			0
	do	1	Twig	15	*a*	27	1	33
	Lilac	1	Leaf	10	*a*	11	1	60
	do	1	do	10	*d*			0
Magnolia (MG-19)	do	1	Petiole	10	*b*	13	1	40
	do	1	Shoot	10	*a*	8	1	70
	Magnolia	1	Leaf	10	*b*	14	1	90
	do	1	do	10	*d*			0
	do	1	Petiole	10	*a*	14	1	70
	Plum	1	Twig	15		24	1	20
	Apple	1	Fruit	20	*a*			0
	do	1	do	25	*d*			0
Apple leaf (A-20)	do	1	Leaf	15	*a*			0
	do	4	do	45	*b*			0
	do	1	Twig	15	*a*			0
	Plum	1	do	10	*a*			0

In 1939 infection was obtained on Rome Beauty, Willow Twig, Jonathan, Stayman, Delicious, Red Astrachan, Red June, Yellow Transparent, Carson, Colton, Early Harvest, and Liveland apples. Infection was not obtained on King David, Winesap, and Arkansas Black. The infection obtained on peach leaves confirmed the results of Dunegan (*12*), who found peach susceptible to blister spot and other bacteria actually introduced into leaves and twigs. Unlike the blister spot organism, *Phytomonas syringae* SI-1 did not infect any of the varieties of apple or cherry.

In 1940 infection was obtained on Rome Beauty, Golden Delicious, Delicious, and Willow Twig apples. Infection was not obtained on Black Twig and Stayman. Apple twigs (fig. 5, *C*) and fruit were also infected by the New York strain of *Phytomonas syringae* and by the

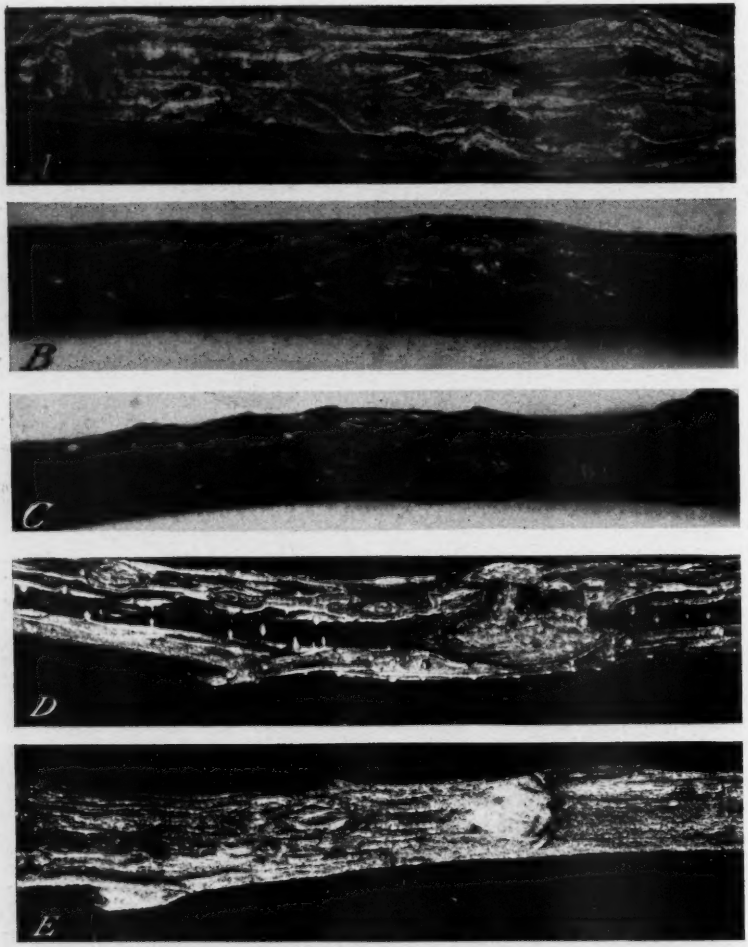

FIGURE 5.—*A* and *B*, Twigs artificially infected with the blister spot organism, × 2: *A*, President plum 25 days after inoculation; *B*, Willow Twig apple 26 days after inoculation. *C* and *D*, Artificial infection of twigs with NY strain of *Phytomonas syringae*: *C*, Rome Beauty apple, × 2; *D*, President plum, × about 3. *E*, Check puncture on President plum, × about 3.

magnolia isolate. Lilac and plum were both infected by blister spot, *Ph. syringae*, and magnolia isolates; magnolia also was infected by blister spot and magnolia isolates (see fig. 5, *A* and *D*).

When lilac leaves were inoculated with the blister spot organism by scratching the surfaces with a hypodermic needle containing a water suspension of the organism, the infection appeared as greenish-yellow lines along the path of the needle scratches. A water-soaked area extending on either side of the scratch was often visible on the fourth or fifth day after inoculation. No infection was obtained when nonwounded leaf surfaces were sprayed with a water suspension of the organism. Infection on lilac shoots was localized, the most pronounced type of lesion being characterized by slightly raised, irregular, purplish spots, often not more than 3 to 4 mm. in diameter. Some spots had water-soaked areas around them. Pure culture re-isolations of the organisms from these lesions were readily made. There was never any indication of wilting or extensive vascular infection such as has been shown by Bryan (*6*) to occur when lilac is inoculated with *Phytomonas syringae*.

Rose (*28*) stated that some strains of *Phytomonas papulans* were infectious to all varieties of apples tested and others to only one or two, and suggested that further work was necessary. In the present tests no clear-cut strain differences were demonstrated.

The highest percentages of infection with the blister spot organism were obtained during May and June and the lowest during July, August, and September; no infections were obtained from inoculations made in April or October. The average incubation period on fruit inoculated with the blister spot organism was 21 days. In general, as expected, the fewest infections were obtained in nonwounded tissue. The highest percentage of infection on twigs was obtained on those that were growing rapidly.

The lesions produced on apple fruits by wound inoculations with *Phytomonas syringae* were slightly irregular, brown to black, water-soaked spots. There was no evidence of the production of a papilla, which is characteristic of the type of infection produced by the blister spot organism. Artificial inoculations of apple fruits performed under the same conditions with the blister spot organism resulted in infections showing characteristic papillae. No infection was obtained with *Ph. syringae* when nonwound inoculations were made on fruits.

INOCULATION OF IMMATURE APPLES AND STONE FRUITS

On June 6, 1939, immature apple, plum, cherry, and peach fruits were removed from trees and inoculated with a water suspension of a 24-hour-old culture of the blister spot organism by making four needle punctures through drops on each fruit. Checks consisted of punctures made through sterile-water drops. The fruits were held in moist chambers at 25° C. for 8 days. Doubtful infections were checked by culturing on beef-infusion-agar plates and any suspected organisms by further inoculations of fruits.

Successful inoculations, varying from 80 to 100 percent, were obtained on 7 apple varieties. These in order of susceptibility were White Pippin, Grimes, Skelton, Ada Red, Polly Eads, Twenty Ounce, and Grays Red. Smaller percentages of infection occurred on 17 apple varieties, as follows: Fanny, Ishewood, Colton, Lansingburg, Summer Champion, Benoni, Victuals and Drink, Early Ripe, Carson, Wealthy, Early Harvest, Sops of Wine, Red June, White Winter Pearmain, Lowell, Carson, and Red Astrachan. Inoculations failed

on 9 varieties (Yellow Transparent, Liveland, Early McIntosh, Duchess, Maiden Blush, Pennock, Henry Clay, Hopa Crab, and Whitney Crab). Indian Blood plum and Yellow Glass cherry were successfully inoculated. Inoculations of Gold cherry and Elberta peach were not successful.

This experiment shows that immature apple (fig. 3, *a*), plum (fig. 6), and cherry fruits may be removed from the trees and successfully inoculated in moist chambers. Since the incubation period is relatively short (5 to 8 days), the method is of value for quick testing of the pathogenicity of the blister spot organism.

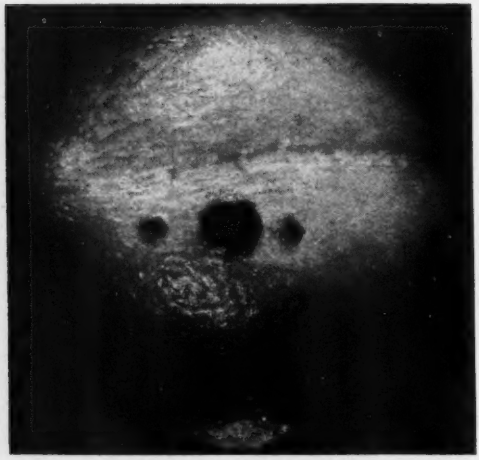

FIGURE 6.—Artificial infection of Indian Blood plum with the blister spot organism, × about 5.

During the 1939 season two attempts were made to induce infection in moist chambers of nonwounded immature fruits of 24 varieties of apples. Ten fruits of each variety were atomized with a water suspension of a 24-hour-old culture of the blister spot organism and held in moist chambers at a temperature of 26° C. Nonwounded fruits atomized with sterile water served as checks. In no instance was there definite evidence that infection had occurred during a 12-day period.

INOCULATION OF TOMATOES

A water suspension of the blister spot organism was introduced by means of needles into 12 mature and 12 immature tomato fruits in moist chambers. Within 3 days water-soaked areas appeared around most of the points of inoculation on the immature fruits, but not on the similarly inoculated mature fruits. When final observations were made 1 week later, no infection was evident on the mature, inoculated fruits or on the check fruits. The diseased areas which occurred at most of the points of inoculation on the immature fruits consisted of black, water-soaked, slightly raised lesions about 2 to 5 mm. in diameter. Reisolations of the organism from such lesions were readily made. Smith and Fawcett (*31*) obtained similar results with the citrus blast organism, *Phytomonas citriputeale*, when they inoculated fruits of tomato a short time before the fruits reddened. Their report does not show that mature tomato fruits were inoculated.

INOCULATION OF LEMONS AND ORANGES

Smith and Fawcett (*31*), Rosen and Bleecker (*30*), and others have tested the infectivity of certain fluorescent bacteria, such as

Phytomonas syringae, *Ph. cerasi*, and *Ph. citriputeale*, by inoculating lemons or oranges and noting the size of lesions produced on them.

The writer has made many inoculations of lemon and orange fruits with isolates of the blister spot organism, *Phytomonas syringae*, and the target canker, magnolia, and apple leaf spot organisms. The *Ph. syringae* isolates have, in most instances, produced a characteristic black pitting on these hosts, and reisolations were readily obtained.

The blister spot isolates have never produced this typical black pitting. However, 8 days after inoculation with this organism the tissue immediately surrounding the needle puncture becomes sunken and somewhat water-soaked in appearance. After 10 days there may be a light-brown discoloration at the point of inoculation and the tissue 1 to 2 mm. beneath the skin in the infected areas has a glassy, water-soaked appearance. A microscopic examination of this area reveals the presence of large numbers of bacteria, and in most instances reisolations yield pure cultures of the blister spot organism.

The magnolia isolate when introduced into lemons and oranges has produced the typical black pitting characteristic of *Phytomonas syringae* inoculations. Successful inoculations of lemons and oranges with the two target canker isolates and the apple leaf spot isolate have not been obtained.

OVERWINTERING OF THE BLISTER SPOT ORGANISM

During the course of these studies numerous isolations from twig and wood cankers and leaves of the previous year have been attempted in an effort to discover how the blister spot pathogen survives the winter. None of these has proved successful. Inasmuch as apple fruit infections are the only known natural manifestation of the disease, it seemed important to study the overwintering of infected fruit.

In October 1939, Rome Beauty apple fruits affected by the blister spot disease were placed on the ground in the orchard and one-fourth-inch mesh wire screen was placed over them as a partial protection against rodents. Beginning October 16 and at approximately 1-month intervals throughout the fall and winter of 1939–40, isolations were attempted by removing the blisters from the apples with aseptic precautions, crushing them in tubes of sterile water, and culturing them on plates of Patel's sodium taurocholate medium (*23*), which was found to be the most satisfactory for this purpose. Table 3 records the results of these studies.

In this experiment the blister spot pathogen remained viable from October 16 to March 15, but no growth occurred in the cultures made in April and May. Fewer blister spots were plated in the winter and spring months because gradual disintegration of the fruits made it difficult to select suitable material.

The apparent failure of the pathogen to remain viable after March 15 seems to indicate that blister spots on overwintering fruits do not constitute a source of infection in the spring. It is obvious that the pathogen would have to survive at least until May 1 to initiate infection on young fruits of the next crop. As these results apply to one season only, they do not wholly preclude the possibility of overwintering of the organism on the fruit.

TABLE 3.—*Overwintering of blister spot pathogen on apple fruits*

Date of culture	Fruits	Blister spots	
		Cultured	Viable
1939	*Number*	*Number*	*Number*
Oct. 16	24	22	14
Nov. 15	17	14	6
Dec. 14	12	15	6
1940			
Jan. 17	6	12	4
Feb. 15	5	5	1
Mar. 15	2	6	1
Apr. 18	1	3	0
May 15	1	1	0

PHYSIOLOGY OF BLISTER SPOT AND RELATED ORGANISMS

In studies of the cultural characters and biochemical reactions of the various isolates, the methods used were essentially those recommended by the Society of American Bacteriologists (*32*). The hydrogen-ion concentration of all the media used was determined by the colorimetric and quinhydrone-electrode methods. All the organisms were cultured in beef-extract-peptone broth (pH 6.8 to 7.0) for 12 hours before being transferred to the media used in the studies. Unless otherwise stated, all cultures were incubated at 25° C. The physiological studies were based on 18 isolates of the blister spot organism, 3 isolates of *Phytomonas syringae*, 2 isolates of the target canker organism, and 1 each of the magnolia and the apple leaf spot organisms.

GROWTH ON VARIOUS MEDIA

BEEF-INFUSION-AGAR SLANTS.—On beef-infusion agar adjusted to a pH of 6.8, the growth of the blister spot organism was visible after 24 hours as a grayish-white, filiform line following the path of the needle. After 3 days the growth was abundant, filiform, glistening, butyrous, and white. After 10 days the mediun was greened. It was observed occasionally that certain of the blister spot isolates which had been in culture for a long time had apparently lost their ability to produce pigment. This feature of the fluorescent plant pathogens was mentioned by Burkholder (*7*). In some isolates the lower end of the slant assumed a pink tinge after 3 weeks.

The growth of *Phytomonas syringae* and of the magnolia isolate was flatter than that of the blister spot organism, but otherwise they exhibited the same characteristics. Target canker isolate T–64 was characterized by abundant, filiform, glistening, grayish-white, somewhat slimy growth with a tendency to darken after 8 days. Greening of the medium was more prompt than with the blister spot organism or *Ph. syringae*. With target canker isolate T–62, the growth was scant, filiform, viscid, and flat, and the medium was not greened. The apple leaf isolate was characterized by abundant, filiform, slimy, glistening, grayish-white growth becoming grayish brown after 10 days. The medium was greened.

BEEF-INFUSION BROTH.—In beef-infusion broth (pH 6.9) the blister spot organism, *Phytomonas syringae*, the magnolia isolate, and target canker isolate T–64 produced abundant clouding in 24 hours. With the blister spot organism green fluorescence began in 3 days, starting at the top. The pellicle was of delicate structure and broke easily upon jarring. The sediment was flaky, and there was no odor. *Ph. syringae* isolates were indistinguishable from the blister spot organism except that there was slightly less clouding. Growth of target canker isolate T–62 was scant, and no pellicle was formed. There was no sediment and no greening of the medium. With the apple leaf isolate growth was abundant, a slight pellicle was formed, and the medium was greened.

BEEF-EXTRACT BROTH.—The growth of the blister spot organism, *Phytomonas syringae*, the magnolia isolate, target canker isolate T–64, and the apple leaf

isolate was the same as in beef-infusion broth except that the pellicles formed were less stable and there was no greening of the medium. Target canker isolate T–62 did not form a pellicle, and there was no greening of the medium.

BEEF-INFUSION-AGAR PLATES.—Colonies of the blister spot organism were visible in dilution plates after 48 hours. They were round, smooth, entire, white, convex, and opalescent; some isolates became concentrically ringed after 5 days (fig. 4, *B*). The medium was greened. Illinois, Netherlands, and New York strains of *Phytomonas syringae* closely resembled the blister spot organism during the first 48 hours of their growth. As a rule, the colonies of *Ph. syringae* were smaller than those of blister spot. The Netherlands strain retained its smooth surface at all times. The Illinois strain became cross-hatched in 4 days. As this strain increased in age, the wrinkled appearance grew more pronounced. The New York strain was indistinguishable from the Netherlands strain. The colonies were smooth with no cross hatching. The colonies of the magnolia isolate were round, slightly raised, and opalescent and had rough edges. The medium was greened. Colonies of target canker isolate T–64 were round to irregular, cream-colored, and convex; the medium was greened. Colonies of target canker isolate T–62 were round, flat, and opaque and had rough edges. Colonies of the apple leaf isolate were white, glistening, convex, and opalescent; the medium was greened.

POTATO-DEXTROSE-AGAR SLANTS.—On this medium isolates of the blister spot organism, *Phytomonas syringae*, and the magnolia isolate showed moderate growth, which was filiform, glistening, and butyrous and had no odor. The lower end of the streak became light brown in 2 to 3 weeks, and in 5 weeks the entire streak had assumed this color. There was no greening of the medium. Target canker isolate T–64 and the apple leaf isolate produced slight to moderate growth, which was filiform, glistening, and butyrous. With target canker isolate T–62, growth was slight, filiform, flat, gray, and viscid. There was no brown discoloration of the medium.

SODIUM TAUROCHOLATE AGAR.—After 24 hours, growth of the blister spot organism, *Phytomonas syringae*, and the magnolia, target canker, and apple leaf spot isolates was only faintly visible on slants of sodium taurocholate agar (pH 6.8) prepared according to the directions of Patel (*23*). After 48 hours, growth of the blister spot organism, *Ph. syringae*, magnolia isolate, and apple leaf spot isolate was moderate, but in 3 days it was abundant. Growth was filiform, glistening, and smooth, with a bluish cast. In sodium taurocholate poured plates, the colonies of the blister spot organism, *Ph. syringae*, magnolia isolate, and apple leaf isolate were round, smooth, entire, convex, and bluish with a deeper blue center. Streaks of the blister spot organism showed heavy rugose growth after 4 days (fig. 4, *C*). Target canker isolate T–62 produced scant growth. The growth of *Staphylococcus citreus* (Migula) Bergey et al., a Gram-positive organism used as a check, was almost completely inhibited in this medium.

BEEF-EXTRACT-AGAR STABS.—In stab cultures of beef-extract agar (pH 6.8) growth of the blister spot organism, *Phytomonas syringae*, and the magnolia, apple leaf, and target canker isolates was slow; at the end of 8 days it was visible for less than one-fourth the length of the needle track. All the isolates produced circular colonies, at first white but later becoming gray, on the surface of the medium at the point of inoculation. There was no greening or liquefaction of the medium in any of the cultures.

BEEF-INFUSION-GLYCERIN AGAR.—On beef-infusion-glycerin agar (pH 6.9) the blister spot organism made rapid growth, spreading over the surfaces of the slant within 48 hours (fig. 4, *D*). The medium assumed a pronounced green fluorescence within 48 hours. *Phytomonas syringae*, the apple leaf isolate, and the target canker isolate T–64 produced moderate growth during a similar period. Growth was filiform, flat, glistening, and butyrous, and there was a slight greening of the medium. Growth of target canker isolate T–62 was filiform, scant, and viscid, and there was no greening of the medium.

ASPARAGIN MEDIUM.—All the isolates were grown in asparagin medium No. 2, as recommended by Georgia and Poe (*14*). Growth was abundant and a greening of the medium was apparent after 48 hours with all the isolates except target canker T–62. The greening of this medium by the apple leaf isolate was very intense.

USCHINSKY'S SOLUTION.—All the isolates clouded this medium after 24 hours, and a pellicle formed after 48 hours. All but target canker T–62 greened the medium after 48 hours and produced abundant white precipitates.

FERMI'S SOLUTION.—All the isolates except target canker T–62 produced moderate growth and greened the medium after 48 hours. A heavy pellicle was produced by the blister spot organism, *Phytomonas syringae*, the magnolia isolate, and target canker isolate T–64.

COHN'S SOLUTION.—This medium supported the growth of only three isolates of the blister spot organism. Where growth was present it was scant and was evidenced by a faint white clouding. Growth of *Phytomonas syringae*, magnolia isolate, and target canker isolate T–64 was evidenced by faint, white clouding. Target canker isolate T–62 did not grow in this medium.

MODIFIED CZAPEK'S MEDIUM.—This medium was prepared according to the formula for Czapek's solution except that tyrosine was used to supply nitrogen and sodium succinate was used to replace the sugar ordinarily added. Fifteen isolates of the blister spot organism, the Illinois and Netherlands strains of *Phytomonas syringae*, and target canker isolates T–62 and T–64 were inoculated into this medium. In 7 days a faint pink discoloration of the medium was evident in the tubes inoculated with the blister spot, *Ph. syringae*, and the apple leaf isolates. In 21 days the media in these tubes were mahogany brown in color. The change in color from pink to mahogany brown was due to enzymic action, in this instance to the formation of tyrosinase. The tubes inoculated with the target canker isolates showed no change in color in 7 days, although growth was moderate, and only a faint pink discoloration in 21 days. The New York strain of *Ph. syringae* and the magnolia isolate were not grown in this medium.

MALACHITE GREEN AGAR.—This medium was prepared by adding to Wilson's basal medium No. 2 (*34*) 10 gm. of dextrose, 14 gm. of agar, and malachite green so as to make a dilution of 1 to 100,000. The pH was adjusted to 6.9. The blister spot organism, *Phytomonas syringae*, and magnolia isolates made good growth, which was flat, butyrous, and opalescent. No pigment was produced. The malachite green color gradually disappeared and at the end of 18 days none remained. Target canker isolates T–62 and T–64 and the apple leaf isolate failed to grow in this medium.

MISCELLANEOUS BIOCHEMICAL REACTIONS

GELATIN LIQUEFACTION.—With the blister spot organism, *Phytomonas syringae*, the magnolia, apple leaf spot, and target canker T–64 isolates, liquefaction of plain Bacto gelatin stabs (pH 6.9) began in 24 to 48 hours. The surface of the medium was at first crateriform, later becoming stratiform. The surface layers showed green fluorescence. Liquefaction was four-fifths complete in 2 weeks and became complete in 4 weeks. In freshly isolated cultures of the blister spot organism complete liquefaction occurred within 3 weeks. Target canker isolate T–62 failed to liquefy gelatin.

HYDROLYSIS OF STARCH.—Streak inoculations were made in beef-extract agar containing 0.2 percent soluble starch. In one series, which included all the organisms, after 5 days' growth the surfaces of the plates were flooded with a saturated solution of iodine in 50 percent alcohol. A second series was similarly treated after 10 days. None showed any clear zones outside the area of growth, indicating that no diastatic action had occurred.

NITRATE REDUCTION.—Tests of the various isolates were made on 1-, 2-5-, 7-, 10-, and 14-day-old cultures. The α-naphthylamine sulfanilic acid test was employed. There was no reduction of nitrate by any of the isolates. When *Escherichia coli* (Migula) Castellani and Chalmers was used as a check organism, nitrites were produced from nitrates.

INDOLE PRODUCTION.—The isolates were tested for indole production on Bacto-tryptophane broth (pH 6.8) by the Ehrlich-Bohme test. Tests made at the end of 7, 12, and 18 days were negative. When *Escherichia coli* was used as a check organism, a positive test for indole was obtained.

AMMONIA PRODUCTION.—Strips of filter paper saturated with a freshly prepared Nessler's solution and hung over 24-, 36-, and 48-hour beef-extract-broth cultures of the different isolates gave a strong ammonia reaction in case of all except target canker T–62.

HYDROGEN SULFIDE PRODUCTION.—Strips of lead acetate paper failed to blacken when hung over beef-extract-broth cultures of the different isolates, indicating that hydrogen sulfide was not being produced. Similar tests were made of the different isolates grown on lead acetate agar slants, but the results were negative. *Escherichia coli* used as the check organism gave a positive test for hydrogen sulfide.

REACTION IN MILK.—Fresh skimmed milk sterilized on 3 successive days for 20 minutes at 100° C. was used in these tests. No acid was produced by any of the isolates. Curd formation was evident in the *Phytomonas syringae* and magnolia isolates. An alkaline reaction was observed in 48 hours. *Ph. syringae* and the magnolia isolate cleared the milk in bands, but the blister spot and the other isolates did not.

REDUCTION OF LITMUS.—Litmus milk was blued and a soft coagulum was formed within 6 days by all the isolates except target canker T–62. Within 2 weeks peptonization had begun. *Phytomonas syringae* and the magnolia isolate progressed downward in bands. Reduction of litmus was complete in 4 weeks with all the isolates except target canker T–62, which failed to reduce it. A dark-blue color was evident in 5 weeks in the remaining cultures, and this color was still present when the cultures were discarded 2 months later. While litmus was reduced and a deep-blue color was at first apparent in the tubes inoculated with the target canker isolate T–64, this color changed to blue green after 2 months.

REDUCTION OF BROMCRESOL PURPLE.—Bromcresol purple indicator, at the rate of 1 cc. of a 1.6 percent alcoholic solution per liter, was added to fresh skimmed milk. The medium was adjusted to pH 7.0 with bromthymol blue and then sterilized at 15 pounds' pressure for 15 minutes. Six days after inoculation with the different isolates there was no indication of any acid production. Within 2 weeks the cultures had assumed a wine-red color with reduction complete. No acid reaction was evident.

TOLERATION OF ACID AND ALKALI.—Tests were made in beef-extract broth with a series of hydrogen-ion concentrations from pH 5.0 to 9.4. Growth of the blister spot, *Phytomonas syringae*, magnolia, apple leaf, and target canker isolates occurred in this medium from pH 5.0 to 9.4, with the optimum growth at pH 7.0.

RELATION TO FREE OXYGEN.—The isolates were grown in Smith fermentation tubes containing 1 percent dextrose broth. Growth took place first in the open arms, later progressing toward the domes. These results indicate that none was strictly aerobic. No gas was produced by any of the isolates.

The results summarized in table 4 show that all the organisms with the exception of target canker T–62 exhibited similar biochemical reactions. It would appear therefore that the miscellaneous media under discussion were of little value in distinguishing species. The data, however, seem to indicate a close similarity between the blister spot, *Phytomonas syringae*, the magnolia, and the apple leaf isolates. The apple leaf isolate and target canker isolate T–64 also closely resemble each other. Motility of the latter organism was not demonstrated (table 1). Target canker isolate T–62 because of its nonmotility and its failure to liquefy gelatin and to produce a greening of beef-infusion medium is obviously unlike any of the others and must be considered an unrelated organism.

TABLE 4.—*Summary of miscellaneous biochemical reactions* [1] *of the blister spot and other organisms*

Organism	Isolate No.	Gelatin liquefaction	Milk				Hydrogen sulfide production	Ammonia production	Nitrate reduction	Indole production	Facultatively anaerobic
			Curd formation	Peptonization	Acid	Alkaline					
Blister spot	[2] 14	+	−	+	−	+	−	+	−	−	+
Phytomonas syringae	SI–1	+	+	+	−	+	−	+	−	−	+
Do	SH–2	+	+	+	−	+	−	+	−	−	+
Do	NY	+	+	+	−	+	−	+	−	−	+
Target canker	T–62	−	−	−	−	−	−	−	−	−	+
Do	T–64	+	−	+	−	+	−	+	−	−	+
Magnolia	MG–19	+	+	+	−	+	−	+	−	−	+
Apple leaf	A–20	+	−	+	−	+	−	+	−	−	+

[1] + indicates reaction; − no reaction.
[2] 17 other isolates of the blister spot organism gave similar reactions.

REACTION TO DRYING, SUNLIGHT, AND TEMPERATURE

RESISTANCE TO DESICCATION.—Loopfuls of an aqueous suspension of the blister spot, *Phytomonas syringae*, magnolia, apple leaf, and target canker isolates were placed on each of 12 sterile cover slips. These were placed in a sterile Petri dish, which was then put in a container from which light was excluded. After 24 hours 4 of the cover slips were taken out and 2 of them were dropped into nutrient broth and the other 2 into melted beef-infusion agar. There was no clouding of the broth, nor was there any growth in the poured beef-infusion plates. This procedure was repeated 3 times with identical results. After 48 hours the procedure was again repeated. There were no clouding of the broth and no growth in the beef-infusion agar. From the results obtained under these conditions it was apparent that these isolates were not viable after a 24-hour desiccation.

EFFECT OF SUNLIGHT.—Heavily seeded beef-infusion-agar plates of the different isolates were placed on crushed ice and exposed to direct sunlight for periods of 5, 10, 15, 25, 30, and 40 minutes. Black paper was placed over half of each glass lid during the exposure. The toxic action of the sun rays was not apparent on the plates of any of the isolates exposed for 5 or 10 minutes. There was, however, a gradual diminution in the number of colonies developing in the plates receiving 15-, 20-, and 25-minute exposures. In the plates exposed for 30 minutes no colonies of the blister spot, magnolia, apple leaf, or target canker T–62 and only a few of *Phytomonas syringae* and target canker T–64 isolates developed. No colonies of any of the isolates tested ever developed on the exposed portion of the plates subjected to direct sunlight for 40 minutes. All the organisms made good growth in the portions of the plates covered with black paper.

TEMPERATURE RELATIONS.—The effect of temperatures on the growth of the various isolates was observed by placing the bacteria on the surface of the beef-infusion agar by means of a platinum loop, incubating the plates at the desired temperatures, and measuring the diameter of the colonies at intervals. The increase in diameter of the colonies of all the isolates was greatest at 27° C. Within 24 hours clouding was visible in beef-infusion broth cultures from 9° to 30°. The minimum temperature for growth in beef-infusion-broth was 3.5° and the maximum was 34.5°. The thermal death point was 52° for the blister spot, *Phytomonas syringae*, magnolia, and apple leaf isolates. The thermal death points of the target canker isolates were not determined.

CARBOHYDRATE FERMENTATION

In the carbohydrate fermentation studies the peptone-free medium described by Ayres, Rupp, and Johnson (*2*) but slightly modified as given by the Society of American Bacteriologists (*32*) was used. Thirty-one carbon sources, as shown in table 5, were used as 1-percent additions to the synthetic basic liquid medium. Before and after adding the carbon source the hydrogen-ion concentration was adjusted to approximately neutral by adding sodium hydroxide. Bromcresol purple was then added to the media as an indicator. Duplicate cultures were made in all cases.

TABLE 5.—*Carbohydrate reactions* [1] *of blister spot and various other isolates*

Organism	Isolate No.	Monosaccharides						Disaccharides				Trisaccharides		Polysaccharides			Alcohols			Glucosides			Organic acids					
		Arabinose	Rhamnose	Xylose	Dextrose	Levulose	Galactose	Mannose	Sucrose	Maltose	Lactose	Trehalose	Raffinose	Melezitose	Starch	Inulin	Dextrin	Glycogen	Glycerol	Mannitol	Dulcitol	Sorbitol	Arbutin	Salicin	Esculin	Citric	Malic	Lactic	Formic	Succinic	Tartaric	Acetic
Blister spot [2]	14	+	0	+	+	+	+	+	+	gr	0	gr	gr	gr	0	gr	gr	gr	+	+	gr	+	0	gr	gr	1	1	1	0	1	0	1
Phytomonas syringae [3]	NY	+	0	+	+	+	+	+	+	gr	0	gr	gr	gr	0	gr	gr	gr	+	+	gr	+	0	gr	gr	1	1	1	0	1	0	1
Target canker	T–62	+	0	+	+	+	+	+	+	gr	0	gr	0	gr	0	gr	gr	0	+	+	gr	+	0	gr	gr	1	1	1	0	1	0	1
Do	T–64	+	0	+	+	+	+	+	+	gr	0	gr	0	gr	0	gr	gr	0	+	+	gr	+	0	gr	gr	1	1	1	0	1	0	1
Magnolia	MG–19	+	0	+	+	+	+	+	+	gr	0	gr	gr	gr	0	gr	gr	gr	+	+	gr	+	0	gr	gr	1	1	1	0	1	0	1
Apple leaf	A–20	+	0	+	+	+	+	+	+	gr	0	gr	gr	gr	0	gr	gr	gr	+	+	gr	+	0	gr	gr	1	1	1	0	1	0	1

[1] + = Acid reaction; − = alkaline reaction; 0 = no growth; gr = growth but no change in reaction.
[2] 18 isolates gave similar results.
[3] Blister spot isolates showed alkaline reaction after 21 days.
[4] 3 isolates gave similar results.

To prevent their breaking down during sterilization, the sugars lactose, levulose, maltose, sucrose, and raffinose were sterilized by filtration. The basic medium was sterilized by heat and a sufficient quantity of filtered solution of the sugar was then added to each tube. The prepared media were held for 1 week at 25° C. to allow contaminates to develop if any were present. The other carbohydrate media were sterilized in the autoclave at 12 pounds' pressure for 15 minutes.

In all the fermentation studies with the sugars, alcohols, and glucosides, change of color from purple to yellow was considered to be the index of fermentation. With these methods such a change could be attributed only to the fermentation of the carbon source added to the basic medium, since no other energy source was present. All the cultures were under observation for 21 days before being discarded.

The media with organic acid sources of carbon were prepared according to Ayres, Rupp, and Johnson's method (*2*), except that the formula was modified by using 0.15 percent acid instead of the amount originally recommended. Before and after the carbon sources were added, the media were adjusted to pH 7.0 by the addition of sodium hydroxide. The criterion for fermentation was the presence of growth as indicated by turbidity and an increase in alkalinity.

The final results of the fermentation tests appear in table 5.

The 31 carbohydrates used in the studies give a very wide range of comparison of the fermentative ability of the different isolates. Among the isolates of the blister spot organism there was some variation in rates of reaction, but not in the ultimate results. The 3 isolates of *Phytomonas syringae* were identical in their reactions.

A comparison of the reactions of the blister spot and *Phytomonas syringae* isolates shows that the blister spot isolates produced an alkaline reaction slowly in glycogen; *Ph. syringae* grew in this medium, but there was no change in reaction. *Ph. syringae* grew in salicin, but again there was no change in reaction; it did not grow in esculin. The blister spot isolates grew and produced an acid reaction in both of these media. They also grew in all of the organic acid media except tartaric acid and produced an alkaline reaction. The *Ph. syringae* isolates failed to grow in formic, tartaric, and acetic acids. With the exception of these few differences, the blister spot and *Ph. syringae* isolates exhibited similar reactions in all the carbohydrate media used.

A comparison of the fermentative reaction of *Phytomonas syringae* and the magnolia isolate shows that they are indistinguishable. As has been shown earlier, these two organisms also exhibit similar morphological, pathological, and cultural characteristics.

Target canker T–64 differed from the blister spot organism by its failure to utilize salicin and esculin. Target canker T–62 differed by its failure to utilize arabinose, sucrose, glycogen, sorbitol, salicin, and malic, lactic, formic, and succinic acids. It is, therefore, obviously unrelated to the target canker isolate T–64 or to any of the other isolates which have been investigated.

The apple leaf isolate differed from the blister spot isolate by its failure to utilize glycogen, salicin, and esculin. It differed from the *Phytomonas syringae* and magnolia isolates in its ability to grow in formic and acetic acids.

DISCUSSION AND CONCLUSIONS

Rose (*28*) concluded from certain pathological and physiological tests that the differences between blister spot and the disease of apple bark which he called scurfy bark, were of degree rather than of kind. He suggested, however, that more work was necessary before the question of relationship could be settled. Rhoads (*25*) stated in 1924 that he could find neither the scurfy bark canker nor the blister spot disease in the orchards at Mountain Grove, Mo., where Rose had carried on his original investigations with the blister spot disease 8 years earlier.

Since, as has been pointed out, the blister spot disease is inconspicuous and may easily be confused with the minute lesions often produced by the apple scab fungus *Venturia inaequalis*, it is the opinion of the writer that the disease may have existed at the Missouri State Fruit Experiment Station, at Mountain Grove, from the time of its discovery in 1916 until it was rediscovered there in 1938.

That phase of the blister problem dealing with the scurfy, or rough, bark disease has been investigated not only by Rose but also by Hopkins [13] in South Africa and by Lacey and Dowson (*20*) in England. Hopkins concluded from his comparative physiological studies that the Elgin organism and the blister spot and scurfy bark organisms, while closely related, were, nevertheless, distinct species. Lacey and Dowson concluded that the bacterial organism which they isolated from cankers on seedling apple trees, designated as the Wisly strain, was identical with Rose's rough bark organism (*Phytomonas papulans*). They assumed that this latter organism was the cause of both blister spot and the scurfy bark disease. To date Rose is the only investigator who has advanced any proof that a relationship exists between blister spot of apple and the bark disease. It appears, in view of the evidence at hand, that the status of the scurfy bark disease is uncertain and that the blister spot disease of apples is the only disease that has been proved definitely to be caused by the organism originally called *Ph. papulans*.

In the writer's studies of two isolates from target cankers, provisionally designated as *Phytomonas papulans* by Roberts (*26*), and in further studies by Wilson (*34*), neither one was pathogenic to any of the apple varieties inoculated. These two isolates differ from each other culturally and physiologically and also from the other isolates studied in this investigation. They are regarded as two distinct species unrelated to the apple blister spot pathogen.

Studies of the magnolia isolate have shown it to be indistinguishable morphologically, culturally, and physiologically from *Phytomonas syringae*. The pathogenicity of isolates from the two hosts is about the same, since they are cross-inoculable on lilac and magnolia. Both are pathogenic to apple fruit and twigs. A review of the literature does not reveal any reference to a bacterial disease of magnolia. Magnolia, therefore, constitutes a hitherto unreported host for *Ph. syringae*.

The isolate from leaf spot on Rome Beauty apple exhibited cultural reactions similar to the blister spot, *Phytomonas syringae*, magnolia, and target canker T–64 isolates, but differed from them and from

[13] See footnote 4.

target canker isolate T–62 in certain physiological reactions. Inoculations of apple fruits and leaves and plum twigs were attempted at various times during the season with this isolate, but none was successful. These cultural differences and its apparent nonpathogenicity are sufficient reasons, in the writer's opinion, to consider it an unrelated organism.

Studies showed that the blister spot organism remained viable on fruits placed outdoors from October 16 to March 15. After that time the fruit tissues had largely disintegrated and reisolations were not successful. It is obvious that the organism would have to survive at least until May 1 to initiate infection on young fruits of the next crop. It is concluded, therefore, that blister spots on overwintered fruits probably do not constitute a source of infection in the spring.

The spread of the blister spot disease from other possible hosts to apple has been considered and is worthy of further study. Such a study should include an investigation of the pathogenicity of the blister spot organism on wild as well as on cultivated hosts such as was made with *Phytomonas syringae* by Van Hall (*15*), by Fawcett, Horne, and Camp (*13*), and by Smith and Fawcett (*31*).

As previously stated, the blister spot pathogen and *Phytomonas syringae* are slightly different in cultural and morphological characters. The two organisms proved to be cross-inoculable on apple and lilac. *Ph. syringae* when inoculated into apple fruits was pathogenic but failed to produce the papillae characteristic of inoculations with the blister spot pathogen. The blister spot pathogen has proved to be pathogenic on lilac foliage and shoots but failed to produce the wilting typical of *Ph. syringae*. Both these organisms were pathogenic when inoculated into apple twigs. From the foregoing studies it is apparent that the blister spot pathogen is very closely related morphologically and culturally to *Ph. syringae*. The differences in pathogenic behavior appear to be mainly in degree rather than in kind.

In attempting a classification of the blister spot pathogen three possibilities have been considered: (1) Retaining specific rank for *Phytomonas papulans*, (2) considering the blister spot pathogen as identical with *Ph. syringae*, and (3) considering the blister spot pathogen as a variety of *Ph. syringae*. It is the writer's opinion that, because of its similarity to *Ph. syringae* morphologically, culturally, physiologically, and pathogenically, *Ph. papulans* should not retain specific rank. On the other hand, it exhibits enough cultural, physiological, and pathogenic differences to justify its separation from *Ph. syringae*. Because of these differences, *Ph. papulans* is considered a variety of *Ph. syringae*. An emended description of the proposed variety follows.

TECHNICAL DESCRIPTION

Phytomonas syringae papulans [14] n. var.

(*Pseudomonas papulans* Rose; *Phytomonas papulans* (Rose) Bergey et al.)

Short rod, 0.8μ to 2.5μ long by 0.8μ wide, usually occurring singly but occasionally in pairs or short chains. Motile by one to several polar flagella; no spores or capsules demonstrated; Gram-negative but stains readily with ordinary bacterial

[14] If Dowson's (*11*) proposed system of classification is accepted the name of this organism would be *Pseudomonas syringae* var. *papulans*.

stains; not acidfast; forms round, white, opalescent colonies on beef-infusion agar; clouds bouillon and forms a pellicle; liquefies gelatin; produces green fluorescence in beef-infusion and asparagin media and glycerin agar; produces mahogany-brown color in modified Czapek media; reduces litmus and bromcresol purple milk; produces ammonia but no indole or hydrogen sulfide; no nitrate reduction; no diastatic action on starch; grows in Uschinsky's and Fermi's solutions but very feebly or not at all in Cohn's solution; produces acid from arabinose, xylose, dextrose, levulose, galactose, mannose, sucrose, glycerol, mannitol, sorbitol, salicin, esculin, but not from rhamnose, maltose, lactose, trehalose, raffinose, melezitose, starch, inulin, dextrin, dulcitol, or arbutin; produces alkaline reaction in glycogen and in citric, malic, lactic, formic, succinic, and acetic acids; no gas production; facultative anaerobe; optimum, minimum, and maximum pH values for growth 7.0, 5.0, 9.4, respectively; optimum, minimum, and maximum temperatures for growth 27°, 3.5°, 34.5° C., respectively; thermal death point 52°.

SUMMARY

The blister spot disease of apples was first described from Missouri in 1916; it was attributed to a bacterium, *Pseudomonas papulans* (*Phytomonas papulans* (Rose) Bergey et al.). The disease has been reported only in the United States, from Missouri, Arkansas, Indiana, Pennsylvania, Virginia, and Illinois, and has been found to occur under natural conditions only on apple fruits.

The disease is first apparent as a blister spot surrounded by a water-soaked area. The lesion may extend 0.2 to 0.4 mm. below the cuticle. Immediately beneath the diseased area a phellogen layer, from 3 to 5 cells in thickness, develops. The bacteria are present intercellularly throughout the region of the papules. In later stages the epidermis over the blister spot becomes black, dies, and breaks loose from the surrounding healthy tissue. The fact that the blisters are small and shallow seems to indicate that the blister spot pathogen is a relatively weak parasite.

The pathogenicity of the blister spot organism to wounded and nonwounded immature apple fruits was demonstrated by field inoculations. The incubation period for fruit inoculations averaged 21 days. Wound inoculations of immature fruits of apple, plum, cherry, and of tomato fruits in moist chambers were successful. Wound inoculations of twigs of apple, cherry, pear, plum, and lilac and of leaves of apple, peach, magnolia, and lilac were also successful.

Needle-puncture inoculations of fruits and twigs of apple with the lilac blight organism (*Phytomonas syringae*) were successful. An isolate from an undescribed leaf spot disease of magnolia proved pathogenic to apple fruit and twigs and to lilac and magnolia foliage. Two isolates from target canker of apples did not prove pathogenic to fruit and twigs of apple. An isolate from an undescribed leaf spot on Rome Beauty apple foliage failed to infect apple foliage, twigs, and fruit.

Studies showed that the blister spot organism remained viable on fruits placed outdoors from October 16 to March 15. After that time the fruit tissues had largely disintegrated and reisolations were not successful. It is concluded that blister spots on overwintered fruits probably do not constitute a source of infection in the spring.

A study was made of the morphology and physiology of 18 isolates of the blister spot organism, 3 of *Phytomonas syringae*, 2 from apple target cankers, 1 from an undescribed leaf spot of magnolia, and 1 from an undescribed leaf spot on Rome Beauty apple foliage. The

blister spot, *Ph. syringae*, and the magnolia leaf spot isolates were found to bear close morphological resemblances. The target canker and Rome Beauty apple leaf spot isolates appeared to be unrelated organisms.

A study was made of the cultural characters of the various isolates on beef-infusion agar and broth, on potato-dextrose, Patel's, glycerin, and malachite-green agars, and in Uschinsky's, Fermi's, Cohn's, and asparagin solutions. Biochemical reactions were studied on gelatin and milk media and on those for the production of ammonia, hydrogen sulfide, and indole, for the hydrolysis of starch, and for the reduction of nitrates. In the fermentation studies 31 carbon sources were utilized.

The results of the studies of the physiology of the various isolates indicated that the blister spot, *Phytomonas syringae*, and the magnolia isolates are very closely related. The target canker and the Rome Beauty apple leaf isolates are regarded as unrelated species.

It is concluded that the blister spot organism, because of its morphological, cultural, physiological, and pathogenic similarity to *Phytomonas syringae*, should not retain specific rank. On the other hand, it exhibits enough cultural, physiological, and pathological differences to justify its separation from typical *Ph. syringae*. Because of these differences, the blister spot organism is considered a variety of *Ph. syringae* and is designated *Ph. syringae papulans* n. var. A description is given.

The isolate from the magnolia leaf spot is considered to be *Phytomonas syringae*, and accordingly × *Magnolia soulangeana* is to be regarded as an additional host for this pathogen.

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U. S. GOVERNMENT PRINTING OFFICE: 1944